

Five-hundred microliter breast cancer cell suspensions were added to the upper side of the inserts at a density of 10×10^4 cells/insert and 750 μ l CM with or without other compounds was added to the lower wells. Cells were incubated at 37° C. for 18-20 hr. Cells that did not migrate through the filters were removed using cotton swabs, and cells that migrated through the inserts were fixed and stained with Hema 3 Stat Pack (Fisher Scientific). The number of migrated cells in 5 fields of view per insert was counted under a light microscope at magnification 10 \times .

[0062] Soft agar colony formation assay. For anchorage-independent cell growth, MDA-MB-231 cells were plated in 0.4% agarose with complete medium supplemented with 50 μ M compound (P1 to P10) on top of a 0.8% agarose base supplemented with complete medium. Cells were maintained for about 2 weeks before staining with p-iodonitro-tetrazolium violet (Sigma-Aldrich, St. Louis, Mo.). Images were captured by using a scanner and the numbers of colonies were counted.

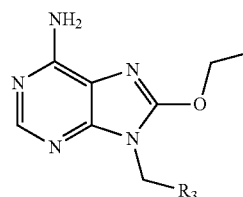
[0063] Animals. Four-week-old female athymic nude mice (Harlan Sprague-Dawley, Indianapolis, Ind., USA) were used for the mammary fat pad injections. Four- to five-week old female C57b1/6 mice were used for the intratibial injections. Animals were maintained under the care and supervision of the Laboratory Animal Research facility at the University of Texas Health Science Center, San Antonio, Tex. The animal protocol was approved and monitored by the Institutional Animal Care and Use Committee.

[0064] In vivo xenograft experiment. MDA-MB-231 cells were injected subcutaneously in the mammary fat pad of 4-week-old female nu/nu athymic nude mice. Each mouse received bilateral subcutaneous inoculation in both the left and right inguinal mammary fat pad areas with 100 μ l of cell suspension containing $\sim 1 \times 10^7$ cells/ml in serum-free media. Animals were randomly assigned to 3 different groups, and solid tumors were allowed to form up to about 5 mm³

volume before treatments began. Compound P3 400 μ mol/500 μ l saline, or saline as a control was administered intraperitoneally (IP) three times a week for 3 weeks. The growth of xenograft tumors was monitored twice a week and tumor size was measured with a caliper in two dimensions. Tumor volumes were calculated with the equation $V = (L \times W^2) \times 0.5$ (mm³), where L is length and W is width of a tumor.

[0065] Statistical analysis. Unless otherwise specified in the Figure Legends, the data are presented as the mean \pm S.E.M. of at least three determinations. Asterisks indicate the degree of significant differences compared with the controls (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). One-way analysis of variance (ANOVA) and Student Newman-Keuls test were used to compare groups using GraphPad Prism 5.04 software (GraphPad).

1. A compound that is a chemical analog of adenosine receptor antagonist 8-Ethoxy-9-ethyl-9H-purin-6-amine with general formula of Formula II



Formula II

where R3 is a heteromethyl, cycloalkyl, or tetrahydrofuran.

2. The compound of claim 1, wherein R3 is difluoro methyl, cyclopropyl, cyclobutyl, or β -tetrahydrofuran.

3. A method for treating a breast cancer or lung cancer patient comprising administering to the patient an effective amount of one or more compounds of claim 1.

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